EB

Week 16

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Addition of BamHI restriction site downstream to the EB sequence and amplification of the whole plasmid, using overhang PCR

Responsible

Aman Mebrahtu

Protocols used

- PCR
- Gel electrophoresis

Experimental Set Up

The purpose of the overhang PCR was to amplify the whole pSB1C3-T7-EB construct with the addition of a BamHI restriction sequence after the EB coding sequence. This BamHI site serves as a place of attachment for linker tags, which will be added later on. To maintain accuracy of the PCR performed, we chose to work with Phusion High Fidelity DNA Polymerase. The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 1: PCR reaction of Overhang EB

Reaction	Volume [μl]
Nuclease-free water	10.8
5X Phusion HF	4.0
10 mM dNTPs	2.0
10 μlM Forward Primer	1.0
10 μlM Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 2: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {°C}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		68	30 secs
Extension		72	1.5 min
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

The PCR was unsuccessful. No clear bands appeared in the gel.

Discussion and Troubleshooting

The PCR program or conditions might not be successful, or the primer might not be compatible.

2 Biofilm assay on pSCB1C3-T7-EB

Responsible

Shuangjia Xue, Jenny Waspe, and Aliki Mitropoulou

Protocols used

Biofilm Assay

Experimental Set Up

Biofilm dispersal by EB was demonstrated using crystal violet assays; one with *P. aeruginosa* as the biofilm-producer and the other with *S. aureus*. Induced BL21(DE3) cell lysate samples (both debris pellet resuspension and supernatant) are used for the experiment. The biofilm is treated with 1 μl, 5 μl, 10 μl, or 20 μl of EB sample. The samples were divided into soluble and insoluble section. Differences between the negative control and EB are evaluated using the students t-test. A p-value ¡0.05 is considered statistically significant.

A crystal assay is conducted to evaluate how EB inhibits biofilm growth by *P. aeruginosa* and *S. aureus*, respectively. Induced BL21(DE3) cell lysate samples (both debris pellet resuspension and supernatant) are used for the experiment.

S. aureus is cultured in BHI media and *P. aeruginosa* PAO in LB media. After an overnight incubation, the cell density was measured at OD 600. 200 μl of the liquid culture is added into each well and treated with EB of four different volumes; 1, 5, 10 and 20 μl. The biofilm is allowed to develop for 48 hours with subsequent staining. OD is then measured again at 600 nm. Cell lysates of BL21(DE3) cells are used as negative control. Differences between the negative control and EB is evaluated using the students t-test. A p-value ¡0.05 is considered statistically significant.

Results and Conclusions

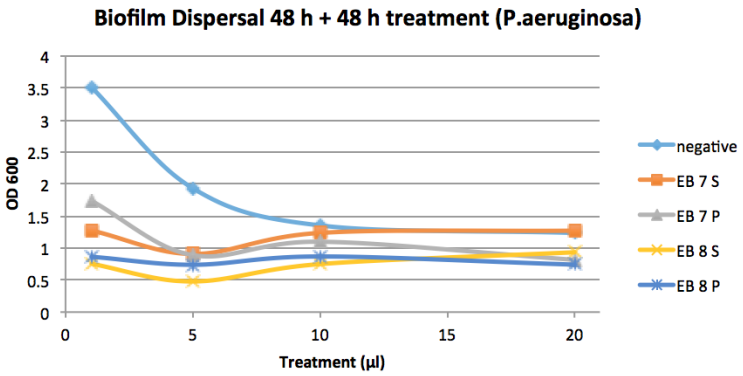

Figure 1: OD measured for different treatments of EB using *P. aeruginosa* as biofilm-producer.

The graph illustrates how different volumes of EB dispersed biofilm formed by *P. aeruginosa*. There is a significant difference between the negative control and of EB 8 treatment. Notably, OD decreases as the amount of negative control increases. This may be due to contamination or elution or dispersal of biofilm because of the increased amount of fluid *per se* in the treatment.

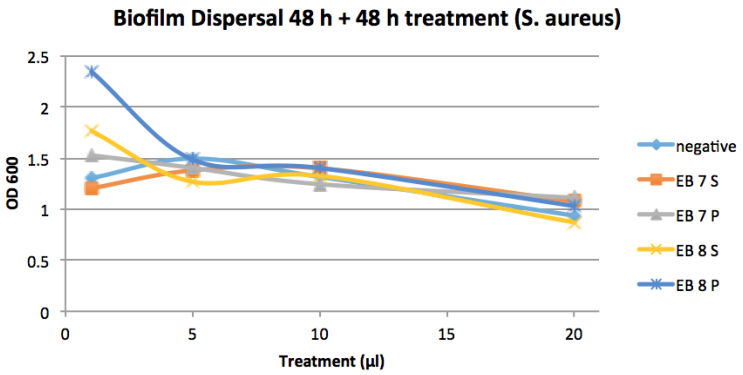

Figure 2: OD measured for different treatments of EB using *S. aureus* as biofilm-producer.

No significant difference was observed in dispersing *S. aureus* biofilm. The biofilm might had regrown at the time point we measured. We could perform another dispersal assay at 4 to 8 hours after treatment.

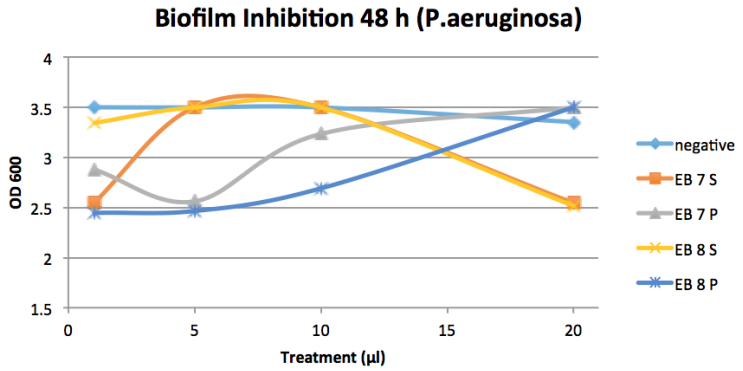

Figure 3: OD measured for different treatments of EB using *P. aeruginosa* as biofilm-producer.

Figure 3 illustrates how the combination of soluble and insoluble section of EB cell lysate inhibits the formation of biofilm by *P. aeruginosa*. EB might have inhibitory capacity, but It's hard to tell from the graph. The dose dependent is hard to observe. 1 μl treatment sometimes works better than bigger volume treatment. This is due to the small sample size, we should increase the sample size in our next experiment.
The graph illustrates how the combination of supernatant and resuspended pellet of EB cell lysate inhibits the formation of biofilm by *S. aureus*. The negative control seems inconsistent; due to the small sample size again. EB 8 showed dose dependent trend for inhibiting *S. aureus* biofilm formation.

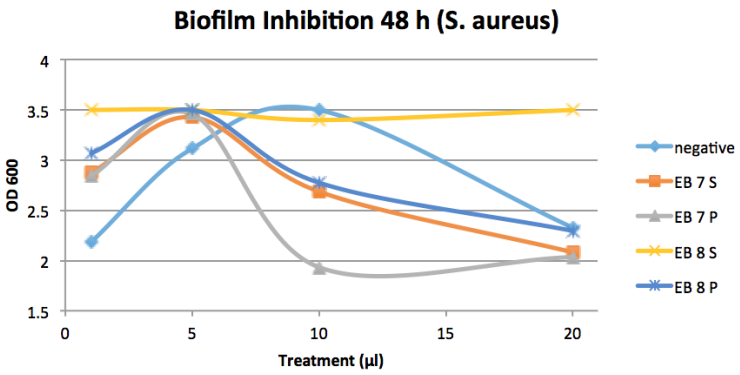

Figure 4: OD measured for different treatments of EB using *S. aureus* as biofilm-producer.

3 Biofilm assay on pSCB1C3-T7-EB - second attempt

Responsible

Shuangjia Xue, Jenny Waspe, and Aliki Mitropoulou

Protocols used

Biofilm Assay

Experimental Set Up

The second attempt of dispersing biofilm was similar to the first. However, this time a combination of pellet and supernatant was used as treatment with the ratio 1:1, e.g. for 5 μl treatment, 2.5 μl of each was used. For this attempt the biofilm was treated with 5 μl, 20 μl and 50 μl of EB. The samples were labelled "EB" and the negative control was labelled "Neg". Only one volume of negative control was used.

The second attempt of inhibiting biofilm growth was similar to the first. However, this time a combination of pellet and supernatant was used as treatment with the ratio 1:1, e.g. for 5 μl treatment, 2.5 μl of each was used. For this attempt the biofilm was treated with 5 μl, 20 μl and 50 μl of EB. The samples were labelled "EB" and the negative control was labelled "Neg". Only one volume of negative control was used.

Results and Conclusions

The figure illustrates how EB dispersed biofilm produced by *P. aeruginosa*. There is a significant difference between the negative control at the highest volumes of EB. The dose dependent trend could be seen in this graph. The volume of treatment influences the dispersal effect, that's why the negative control has lower absorbance than 5 μl EB treatment.
The figure illustrates how EB dispersed biofilm produced by *S. aureus*. The effect is significant difference between the negative control only at the highest dose.

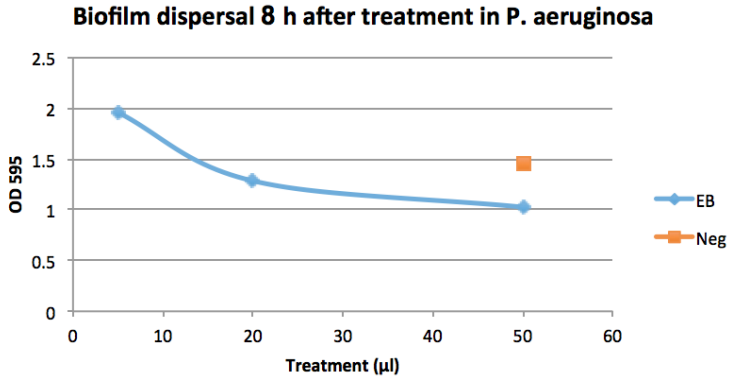

Figure 5: OD measured for different treatments of EB using *P. aeruginosa* as biofilm-producer.

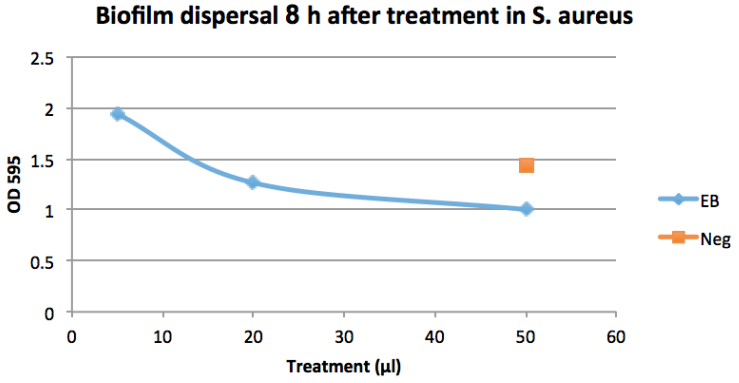

Figure 6: OD measured for different treatments of EB using *S. aureus* as biofilm-producer.

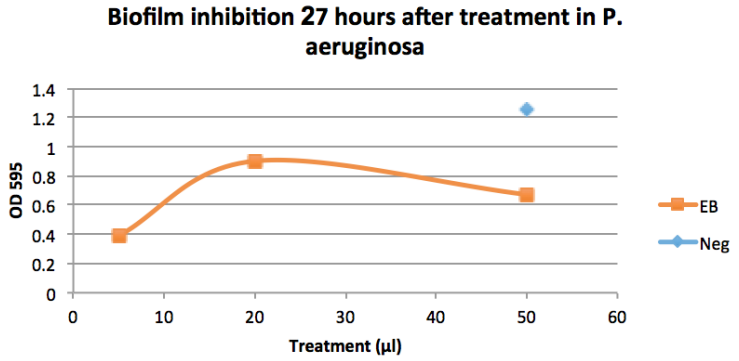

Figure 7: OD measured for different treatments of EB using *P. aeruginosa* as biofilm-producer.

The figure illustrates how EB (supernatant and pellet in a 1:1 ratio) inhibits biofilm formation by *P aeruginosa*. These results show that OD decreases as the volume of treatment increases.

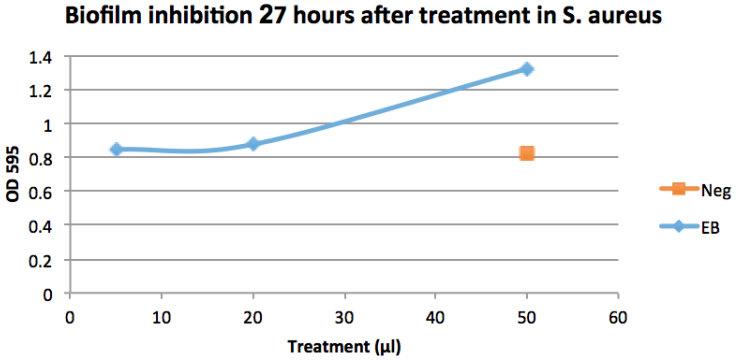

Figure 8: OD measured for different treatments of EB using *S. aureus* as biofilm-producer.

The figure shows EB (supernatant and pellet in a 1:1 ratio) couldn't inhibit biofilm formation by *S. aureus*. The absorbance is similar to negative control, no dose dependent could be seen.